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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Takahiko, ISHIGURO, et al.

Appln. No.: 09/345,761

Confirmation No.: NOT YET ASSIGNED Group Art Unit: 1655

Filed: July 01, 1999 Examiner: WILDER, C

For: METHOD OF ASSAY OF TARGET NUCLEIC ACID

AMENDMENT UNDER 37 C.F.R. § 1.111

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Office Action dated May 23, 2001, please amend the above-identified application as follows:

IN THE CLAIMS:

Please cancel claims 31, 35 and 49 without prejudice or disclaimer.

Please enter the following amended claims and prior claims not presented double spaced:

30. A method for assaying for a specific nucleic acid sequence that is within a target RNA, wherein said target RNA is a single-stranded RNA, said method comprising the following steps:

(1) providing a single-stranded RNA comprising said specific nucleic acid sequence;

(2) hybridizing said target RNA to a reagent (A), which is a single-stranded oligo nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific nucleic acid sequence that is within the target RNA,which allows the target RNA to be cut at the 5' end of the specific nucleic acid sequence by the action of a reagent (D), which is a ribonuclease that degrades RNA in a DNA-RNA double-strand;

(3) cutting the target RNA at the 5' end of the specific nucleic acid sequence to give a product;

(4) hybridizing to said product of step (3), a reagent (B), which is a first single-stranded oligo DNA primer complementary to a sequence at the 3' end of said specific nucleic acid sequence;

D1

(5) extending said first single-stranded oligo DNA primer to the 5' end of the specific nucleic acid sequence with a reagent (C), which is an RNA-dependent DNA polymerase and with a reagent (E), which is deoxynucleoside triphosphates, to form a DNA-RNA double-strand;

(6) digesting the RNA strand of said DNA-RNA double-strand from step (5) with the reagent (D), to give a single-stranded DNA complementary to said specific nucleic acid sequence;

(7) hybridizing to said single-stranded DNA from step (6) a reagent which is a second single-stranded oligo DNA primer having the following sequences, in the following order, beginning at the 5' end and proceeding in a 5' to 3' direction: i) a promoter

sequence for a DNA-dependent RNA polymerase, ii) an enhancer sequence for said promoter sequence, and iii) a sequence at the 5' end of said specific nucleic acid sequence;

(8) extending said second oligo DNA primer to the 5' end of said single-stranded DNA with a reagent (G), which is a DNA-dependent DNA polymerase and with said reagent (E);

(9) synthesizing a single-stranded RNA from said promoter sequence with a reagent (H), which is a DNA-dependent RNA polymerase and a reagent (I), which is ribonucleoside triphosphates;

D1

(10) either:

(a) cycling said single-stranded RNA from step (9) to step (4), or

(b) hybridizing to said single-stranded RNA from step (9) a reagent (J), which is a single-stranded oligo DNA complementary to said specific nucleic acid sequence, labeled so that it gives off a measurable fluorescent signal upon hybridization with a nucleic acid containing said specific nucleic acid sequence; and

(11) after addition of reagents (A) to (J), measuring at least once a fluorescent signal from said hybrid formed in step (10) (b);

wherein said reagents (A) to (J) are added to a reaction vessel one by one, in functional combinations, or all at once.

32. The method according to Claim 30, wherein the reagent (A) is a DNA, and the method further comprises adding an RNaseH and deactivating the RNaseH by heating or by addition of an inhibitor prior to addition of the reagent (B).

33. The method according to Claim 32, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (I), and then by addition of the reagent (J).

D2

34. The method according to Claim 32, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (J).

36. The method according to Claim 30, wherein the reagent (C), an RNA-dependent DNA polymerase, is also the reagent (D), a ribonuclease that degrades RNA in a DNA-RNA double strand.

D3

37. The method according to Claim 30, wherein an enzyme having both an RNA-dependent DNA polymerase activity and a DNA-dependent DNA polymerase activity is used as both the reagents (C) and (G).

38. The method according to Claim 37, wherein the enzyme is avian myoblastoma virus polymerase.

39. The method according to Claim 30, wherein the first and second oligo DNA primers as the reagents (B) and (F) are used at concentrations of from 0.02 to 1 μM.

40. The method according to Claim 30, wherein the DNA-dependent RNA polymerase as the reagent (H) is at least one enzyme selected from the group consisting of phage SP6 polymerase, phage T3 polymerase, and phage T7 polymerase.

41. The method according to Claim 30, wherein the single-straned oligo DNA as the reagent (J) is a DNA which is linked to a fluorescent intercalative dye so that the fluorescent intercalative dye changes its fluorescence characteristic upon hybridization of the DNA with another nucleic acid by intercalating into the resulting double strand.

42. The method according to Claim 30, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

43. The method according to Claim 41, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

44. The method according to Claim 30, which further comprises a step of detecting or quantifying the single-stranded RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

45. The method according to Claim 30, wherein all the reagents are chloride-free.

S46 E3

46. The method according to Claim 30, wherein prior to said step (10)(b)acetate is added as a reagent.

47. The method according to Claim 46, wherein the acetate is magnesium acetate at a concentration of from 5 to 20 mM or potassium acetate at a concentration of from 50 to 200 mM.

D3

S46 E4

48. The method according to Claim 30, wherein prior to said step (10)(b) sorbitol is added as a reagent.

50. The method according to Claim 30, wherein the temperature is selected from the range of from 35 to 60°C.

D4

REMARKS

After entry of the Amendment, claims 30, 32-34, are all the claims pending in the application.

Attached to the Office Action the Examiner returned PTO Form 1449 filed with the Information Disclosure Statement filed May 3, 2001. However, the Examiner did not initial the U.S. Patents listed on the form.

Accordingly, Applicants provide the Examiner with a second copy of the PTO Form 1449. The Examiner is requested, respectfully, to initial the U.S. patents not already considered, thereby indicating they were considered.

Claim Objections

The Examiner pointed out that an Amendment should be double spaced.

In response, Applicants include in this response a new set of claims 30-50, wherein the text is double spaced.

Claim Rejections - 35 U.S.C. § 112

In paragraphs 4.(a)-(d) bridging pages 2 and 3 of the Office Action, the Examiner rejected claims 30-50, all claims remaining, under 35 U.S.C. § 112, second paragraph, as being indefinite for the following reasons.

(a) The phrase "first sequence" in claim 30, step (2) has no antecedent basis.

(b) The description in claim 30, step (2) of "a reagent (A) which allows the single-stranded RNA to be cut" is unclear as to whether reagent (A) actually cuts the single-stranded RNA or whether the feature of "cutting" is only a property of reagent (A).

(c) In claim 30, step (2), the word "exposing" is unclear. The Examiner believes that the term "exposing" is not a positive method step.

(d) The claims are confusing because it is unclear whether reagent (A) operates to cut a single-stranded RNA when reagent (A) is a single-stranded oligo nucleic acid ribozyme or DNA enzyme, because these moieties do not cut RNA.

The Examiner's rejections have been overcome by:

(1) amending claim 30 to refer to "target RNA" rather than simply "single-stranded RNA;"

(2) changing the word "exposing" in claim 30, step (2) to "hybridizing"; and

(3) incorporating the recitation of claim 31 into claim 30, and amending step (2) in claim 30 to recite how the reagent (D) operates.

Claim Rejections -35 U.S.C. § 103

In paragraphs 5 through 9 of the Office Action, the Examiner rejected various claims under 35 U.S.C. § 103(a) over Davey et al., in view of various other references. Claim 31 was not rejected.

Claim 30 has been amended to include the recitation of claim 31. Accordingly, resonciseration and removal of the rejection is requested, respectfully.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

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